Title: A 6,500-year-old Middle Neolithic child from Pollera Cave (Liguria, Italy) with probable multifocal osteoarticular tuberculosis

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Abstract

Clear skeletal evidence of prehistoric tuberculosis (TB) is rare, especially in children. We describe and differentially diagnose the pathological changes displayed by a five-year-old child, Pollera 21 (PO21) dated to the Middle Neolithic of Liguria (Italy), or 5740 ± 30 BP (Beta-409341; 6635–6453 cal BP, 2σ, OxCal 4.2). PO21 shows a number of osteoarticular lesions, mainly of a lytic nature with very little bone proliferation: the vertebral column, the shoulder and pelvic girdles, and the ribcage are involved. Given the nature and pattern of the lesions, we propose a diagnosis of multifocal (or multiple) bone TB. Attempts to detect TB aDNA through molecular analysis gave negative results, but this alone is not sufficient to prove that PO21 was not infected with TB. The lesions observed in PO21 share similarities with other published evidence, such as spinal and joint involvement, and disseminated cyst-like lesions. Conversely, PO21 does not show diffuse bone deposition, such as hypertrophic osteoarthropathy (HOA) or endocranial modifications such as *serpens endocrania symmetrica* (SES). PO21 adds to our knowledge of patterns of TB manifestation in archaeological skeletal remains, which is especially important considering the variability in types and patterns of osteoarticular lesions seen today in people with TB.

Keywords

Paleopathology, disseminated tuberculosis, skeletal tuberculosis.

1. Introduction

Molecular studies suggest a long-standing coexistence of our species with bacterial organisms of the *Mycobacterium tuberculosis* complex (MTBC), although estimates for the coalescence of the MTBC lineages vary in an order of magnitude from thousands to tens of thousands of years (Brosch et al., 2002; Hirsh et al., 2004; Hershberg et al., 2008; Wirth et al., 2008; Comas et al., 2013; Bos et al., 2014). It is mainly the human and bovine forms that affect humans, which are transmitted via the respiratory and gastrointestinal routes, respectively (O'Reilly and Daborn, 1995). Despite this long relationship, TB can only in part be detected in the bioarchaeological record (e.g. see an overview in Roberts and Buikstra 2003: Chapters 3 and 4). In fact, the disease becomes more common – and therefore more likely to be bioarchaeologically detectable – in the Neolithic, as population density increased and people started to live in permanent urban settlements supported by a farming economy (Hershkovitz et al., 2015).

Skeletal evidence of the disease has been documented in human skeletal remains in both the Old and New Worlds (e.g. Buikstra, 1981; Klaus et al., 2010; Roberts, 2015). In the Neolithic Old World, skeletons with the infection have been reported in the Near East and North Africa (Ortner, 1979, 1999; Strouhal, 1987; El-Najjar et al., 1997; Crubézy et al., 1998; Zias, 1998; Dabernat and Crubézy, 2010), with the potential Israeli evidence dating back to the eighth millennium BC (Hershkovitz et al., 2008). Skeletal TB has also been reported from Neolithic continental Europe (Bartels, 1907; Sager et al., 1972; Dastugue and de Lumley, 1976; Bennike, 1999; Gladykowska-Rzeczycka, 1999; Nuorala et al., 2004; Masson et al., 2013, 2015; Köhler et al., 2014; Posa et al., 2015). In Italy, Neolithic evidence of skeletal TB has been reported in the area of Finalese (Finale Ligure, western Liguria), where a number of caves have been excavated since the mid-19th century (Maggi 1997a, 1997b). For example, Formicola et al. (1987) have described an adolescent of about 15 years old with a diagnosis of spinal TB from Arene Candide Cave (Arene Candide 5, Bernabò Brea's excavations; Parenti and Messeri, 1962). The skeleton was recently directly dated to 6570 ± 35 BP (KIA-28340, Le Bras-Goude et al., 2006). An adult Neolithic skeleton with TB in the spine was also reported in an individual from Arma dell'Aquila (number 1 from Zambelli's excavations; Canci et al., 1996), a rock shelter in the Finalese area (5800 ± 90 BP GrN-17730, and more recently via AMS 6318 ± 33 BP, OxA-V-2365-36; Paolo Biagi, personal communication 2016).

We report here a newly analyzed skeleton from the same area and period as Arene Candide 5 and Arma dell'Aquila 1: a young child from the Pollera Cave may have suffered multifocal osteoarticular TB. Clear Neolithic evidence for osteoarticular TB in children has been very rarely reported in the bioarchaeological record. This may be explained in a variety of ways, including differential funerary treatment for children in the past and thus remains being "invisible" (e.g. Bickle and Fibiger, 2014), differential preservation of juvenile skeletal remains (Bello et al., 2006), or a different level of virulence of the infecting MTBC strain or host resistance in the past which would likely have killed individuals before they could develop skeletal changes (Palkovic, 1981; Wood et al., 1992). However, lesions attributed to TB have been described in children from the Roman period in Britain (Lewis, 2011) and Hungary (Hlavenkova et al., 2015), showing that the lesions of TB can be recognized in the archaeological record of children. The most convincing skeletal evidence is described in a 4–5-year-old individual with Pott's disease from Predynastic Upper Egypt (3,200 BC; Dabernat and Crubézy, 2010). The lesions described in a 12-month-old individual from Israel (9,000 BP, Hershkovitz et al., 2008, 2015) have also been attributed to TB based on the molecular detection of the pathogen. However, care should be exercised when linking TB positive aDNA evidence to a non-pathognomonic bone change (Wilbur et al., 2009; but see Donoghue et al., 2009). New evidence of a Neolithic child showing osteoarticular manifestations of TB is therefore important for contributing to our understanding of bone changes due to TB in children's skeletons. In addition, this evidence adds to the overall paleoepidemiological characterization of the disease in the Neolithic, i.e. around the time when it first appears in the remains of people excavated from the archaeological record. Moreover, it could help in identifying non-pathognomonic lesions, or at least patterns of lesions and non-specific bone reactions that could be considered "typical" of disseminated TB infection.

2. Materials and Methods

2.1 Materials

Pollera Cave is situated within a large karstic area near the village of Montesordo in the Finalese area of the western Liguria region (Finale Ligure, Italy; Figure 1). Like other caves nearby (Arene Candide, Arma dell'Aquila, Bergeggi, and several others lie within a radius of a few kilometers), it was excavated during the second half of the 19th century due to the richness of its archaeological deposits that range in date from the Early Neolithic to the Roman period (De

Pascale, 2008). Most of the skeletal remains from Pollera derive from the early excavations, including at least 43 burials now dispersed in various museums, which means that they have suffered from a lack of systematic documentation. However, more recent excavations have made it possible to reconstruct a stratigraphy for the site, which includes Early and Middle Neolithic layers (Tinè, 1973; Odetti, 1990). Skeleton PO21 belongs to a largely complete juvenile individual, which is in a good state of preservation (Figure 2, high-resolution version of the image in S1-1).

Figure 1 and 2 about here

The skeleton was unearthed with at least 15 others during the excavations of G.B. Rossi (1885–1892), and was first mentioned by Issel (1908: 337–338). It was indicated as "probably Neolithic" due to the inclusion in its grave of an eclogite polished stone axe and flint tools. The skeleton was dated via AMS to 5740 ± 30 BP (6635-–6453 cal BP at 2σ with OxCal 4.2; Beta-409341). This range falls within the Middle Neolithic for the area, when the "Squared Mouth Pottery" culture was diffused throughout Liguria (Maggi, 1997a). The previous age at death estimate of about nine years was also revised (Parenti and Messeri, 1962), which was clearly incorrect; based on dental development and eruption patterns (Liversidge et al., 1998; see also Figure S1-2), PO21 was about five years old at the time of death The skeleton is now on display at the Museo di Archeologia Ligure in Genova Pegli (Italy).

2.2 Methods

In order to investigate the lesions apparent in PO21, the skeleton was subject to detailed macroscopic observation; any evidence of abnormal bone formation and destruction was recorded and described, with images taken of relevant areas of the skeleton. In addition, computed tomography (CT) was applied to most skeletal elements (BIOMEDICAL s.r.l., Genova Voltri).

Biomolecular analyses were performed on a rib and a manual phalanx to detect TB infection based on MTBC ancient DNA (aDNA). Ancient DNA was extracted from each bone and subsequently analyzed via polymerase chain reaction (PCR) assays using targets that are supposedly specific to the MTBC (details on the protocol used for aDNA analysis in S2).

3. Results

Pitting and porosity in both orbital roofs (cribra orbitalia) were observable, as well as bone deposition on the right supero-medial orbital surface (the left orbit shows encrustations of

plaster, see Figure S1-3), but no trace of enamel hypoplasia is present in the deciduous dentition. Several lytic lesions and a few areas of bone proliferation were present in the postcranium (visual summary in Figure 3).

Figure 3 about here

3.1 Shoulder girdle

The most obvious abnormality was observed in the left humerus, which is significantly shorter (15 mm) than the right (Figure 2 and S1-4). The distal portion appears normal, while the diaphysis is gracile, with the diameter of the cross-section not increasing mid-distally to proximally, as would be the norm (Bräuer, 1988:199-200). Periosteal remodeling is present in the left diaphysis, with diffuse bone resorption (Figure S1–4). In the proximal metaphysis, new bone formation is present. The proximal epiphysis has a concave aspect and its surface is completely destroyed (Figure 4).

Figure 4 about here

The right scapula displays diffuse pitting on the inferior surface of the scapular blade (ventral aspect). This becomes more marked close to the inferior margin, with two foci of destruction of the cortical surface (one is partially covered by plaster, Figure 5).

Figure 5 about here

3.2 Vertebral column.

One cervical vertebral body (C4) shows clustered pitting and cavitation of its inferior surface (Figure 6). Circular lesions are present on the superior surfaces of the vertebral bodies of T3 (Figure S1-5) and T9 (Figure S1-6); cortical resorption around the margins of these lesions is particularly apparent in T9. The same vertebral body also shows a larger cavity on its inferior aspect and laterally (right side), which has almost completely destroyed the trabecular bone (Figure 7). The vertebral body of T8 was not affected, but its inferior articular facets show clustered pitting, especially on the left side (Figure S1-7).

A small compression fracture is present affecting the center of the inferior surface of L3 (Figure S1-8). Bone resorption is apparent alongside the margins of this lesion, indicating its *in vivo* origin (Figures S1-9 and S1-10). The fifth lumbar vertebra shows a large v-shaped lesion on its inferior surface, which has destroyed part of the vertebral body (Figures S1-11 and S1-12), and partially affected the neural arch. The fifth sacral vertebra presents diffuse porosity and a lesion on the superior aspect of the body (Figure S1-13).

3.3 The rib cage.

The appearance of the ribs is generally normal, except for the sternal ends of four right ribs which show diffuse and clustered pitting, bone resorption with periosteal cavitation, circumferential lesions (possibly cloacae, Figure 10), and small areas of bone deposition on their visceral surfaces (Figures S1-14 and S1-15).

3.4 Pelvis.

Diffuse pitting is present on the ventral aspect of both ilia, especially on the iliac tuberosity (Figure S1-16). In addition, a large lytic lesion with active resorptive margins (10 mm diameter, c. 5 mm deep) is present on the supero-anterior part of the right auricular joint surface (Figure S1-16). The left ischial tuberosity shows diffuse pitting and a circular lesion with cavitation (Figure S1-17). In the right ischium, a concave circular area of bone resorption is present in place of the ischio-pubic ramus (Figure 11).

3.5 Ancient DNA Analyses

None of the PCR assays gave positive amplification results for the MTBC targets screened for, and the possibility that negative results are due to inhibition was excluded (details in S2).

4. Differential diagnosis and discussion

4.1 Differential diagnosis

Pollera 21 was certainly affected by a systemic disease that caused a number of lesions in various regions of the skeleton, mainly of a lytic nature with very little bone proliferation. The diagnosis of skeletal TB is not a simple matter even today in a clinical setting (Aufderheide and Rodríguez Martin, 1998:133; Prapruttam et al., 2014; Ye et al., 2016), and is definitely challenging and prone to errors in a bioarchaeological setting (Wilbur et al., 2009; Roberts et al., 2009). An analysis based on dry bone must consider all possible diagnoses, and there are clearly lesions that may have been caused by both TB and other conditions in this skeleton.

Bone changes of the shoulder girdle, humerus, and vertebral column could have been caused by non-tuberculous infectious/pyogenic/septic arthritis/spondylitis (Resnick and Niwayama, 1995: 2484; Resnick and Kransdorf, 2005: 764; Singh et al., 2009; Jagdap et al., 2013). Both tuberculous and non-tuberculous infectious arthritis only usually affect either the left or right joint of a corresponding pair. However, the progression of pyogenic arthritis is rapid and with abundant bone formation (Resnick and Niwayama, 1995: 2484; Resnick and Kransdorf,

2005: 764). In PO21, bone formation is minimal and diffuse resorptive activity predominates. In addition, the reduced length and the lack of subperiosteal bone apposition apparent in the left humeral diaphysis suggest a period of compromised skeletal development (Sparacello et al., In Press). Non-tuberculous pyogenic spondylitis is difficult to distinguish from tuberculous spondylitis, even in clinical situations, and they often require microbiological and histopathological examination. However, contrary to what was observed in PO21, usually pyogenic spondylitis affects two or fewer lumbar or thoracic vertebrae, and the destruction of more than half of a vertebral body is infrequent (Huang, 1996; Lee, 2014).

Many fungal infections can become systemic and mimic TB, and they are often impossible to distinguish from TB without molecular data, soft tissue changes and clinical data about the patient. However, some mycoses are endemic in certain regions today (e.g., blastomycosis, coccidioidomycosis, and histoplasmosis are limited or more common in North America), to the exclusion of Italy (Rivasi et al., 2000), assuming that the geographic distribution of diseases has remained the same through time. They also preferentially affect selected parts of the skeleton, or result in perifocal bone proliferation (Harrison et al., 1991; Shadomy, 1981; review and references in Aufderheide and Rodríguez Martin, 1998: 212–222). For example, actinomycosis can affect the neural arches, but it primarily affects the mandible, and bone involvement is nevertheless rare.

Tumors can affect bones, and multiple bone metastases have been reported to resemble disseminated osteoarticular TB (Ye et al., 2016). Although cancer in children is rare, primary bone sarcoma is more common than in adults (Holden et al., 2015: 11). However, the disease is typically expressed by large lytic and sclerotic lesion with “fluffy” or cloud-like osteoid production and an aggressive periosteal reaction (Holden et al., 2015: 19). Among benign tumors predominantly affecting children, Langerhans cell histiocytosis (LCH), often referred to as eosinophilic granuloma (EG), produces solitary or multifocal lesions that may resemble those observed in PO21’s pelvis and ribs, and can affect the spine, albeit rarely (Holden et al., 2015: 27; Grenlee et al., 2007). However, most cases are solitary lesions, and in multifocal cases the involvement of the cranium, mandible, and femoral and tibial diaphyses becomes common (McCullough, 1980).

The bacterial infection brucellosis is endemic in Italy and is transmitted through the ingestion of milk products containing the bacteria, or via contact with infected animals, such as

cattle and sheep (Lifeso et al., 1985; Resnick and Kransdorf, 2005: 756). Bone involvement is relatively uncommon, and tends to affect one joint, especially the hip and knee (Lusbani et al., 1986; Benjamin et al., 1992; Hassanjani et al., 2003; Pourbagher et al., 2006). However, brucellar spondylitis causes vertebral damage that can be easily confused with TB (Bodur et al., 2004; Resnick and Kransdorf, 2005: 756). Nevertheless, radiographs of affected vertebrae show dense sclerosis around and beneath lesions due to reparative attempts early in the course of the disease, known as the "Sign of Pedro-Pons" (Pedro-Pons and Farreras, 1944; Exteberria, 1994). Radiographic analysis of PO21, including all the vertebral bodies, excluded such occurrences (results not shown here). In addition, brucellosis is sometimes accompanied by new bone formation on long bones (Roberts and Buikstra, 2003: 96). For the lesions of the pelvis, sacroilitis secondary to brucellosis is a possibility but it is often accompanied by sclerotic bone deposition (Bodur et al., 2004; Pourbagher et al., 2006), which was not observed in PO21.

Differential diagnoses could be made for some of the lesions if they were found in isolation, such as pneumonia, chronic bronchitis, neoplastic disease and blastomycosis for rib lesions (Roberts and Buikstra, 2003: 105). Taking into account the overall nature and pattern of the lesions, it is proposed that they are most compatible with a diagnosis of multifocal (or multiple) bone TB (Cremin et al., 1970). This is a condition described especially in children as multiple cystic or pseudocystic TB (e.g. Malik et al., 2009). The reliability of the diagnosis for PO21 is enhanced because of involvement of the spine, which is the most commonly affected part of the skeleton (Luk, 1999: 338; Roberts and Buikstra, 2003: 95, and references therein). As expected, based on clinical evidence, the lower thoracic and lumbar spinal bodies of PO21 were most affected (Nathanson and Cohen, 1941; Resnick and Niwayama, 1995: 2463). However, the vertebral column of PO21 also shows cervical and sacral spine involvement, which is not common (Resnick and Niwayama, 1995: 2463). Similarly, the involvement of the neural arches observed is rare, although there are reports both clinically and bioarchaeologically (Buikstra, 1976; Kelley and El-Najjar, 1980; Kumar, 1985).

Lesions in the humerus and the scapula may be due to tuberculous arthritis secondary to adjacent osteomyelitis caused by TB (Resnick and Niwayama, 1995: 2462; Resnick and Kransdorf, 2005: 763). In children, the joints are more rarely affected than in adults, but involvement of the epiphysis and metaphysis of the proximal humerus has been reported clinically (Sorrel and Sorrel-Dejerine, 1932:165–168; Kant et al., 2006). Tuberculosis can also

destroy the growth plate – as might have happened if the bone changes are due to TB in PO21 – with potential consequent growth deficit and/or deformity of the bone (Roberts and Buikstra, 2003: 97). This can cause osteopenia proximal and distal to the affected joints, marginal erosion of bone, and destruction of subchondral bone (Aufderheide and Rodríguez Martin, 1998: 138). As observed in PO21, only the cortex overlying the lesion has responded with marked reactive bone formation (Figure 226 in Sorrel and Sorrel-Dejerine, 1932). Tuberculosis of the scapula is described as extremely rare in the clinical literature, although it is more common in areas where the disease is endemic, and is usually associated with other forms of tuberculous osteomyelitis (Srivastava and Srivastava, 2006; Jain et al., 2009; Singh et al., 2009; Jagtap et al., 2013).

The ischium and ischio-pubic ramus is a rare site for TB, but it is seen most commonly in childhood (Blankoff, 1927; Moon et al., 1990; Garcìa et al., 1994). Sacroiliac joint involvement occurs in up to 2% of people with skeletal TB, and is commonly bilateral. Involvement of the ilium and sacroiliac joint is usually considered as secondary to Pott's disease with a psoas abscess, or gastrointestinal TB, and is usually not encountered until late in childhood or even into young adulthood (Kremer and Wiese, 1930; Sorrel and Sorrel-Dejerine, 1932: 501; Konschegg, 1934; Aufderheide and Rodríguez Martin, 1998: 139; Roberts and Buikstra, 2003: 98; Bali et al., 2010).

When the specific lesions are observed in detail, they conform to the general pattern of bone and joint TB, at least for the changes observable in dry bone (Buikstra, 1976). Large sequestra are absent, and PO21 displays very little perifocal reactive new bone formation; the lesions are primarily or exclusively lytic. The exposed cancellous bone is characterized by increased trabecular separation and thickened trabeculae, suggesting a process of damage and repair (Figure 4; see also Figure 2 in Coqueugniot et al., 2015). In the long bones, in this case in the humerus, the diaphyses are not involved, and lytic processes are localized in the epiphyses. Several lesions appear to have characteristics that suggest their association with a granulomatous inflammatory process, with surface cavitations surrounded by clustered pitting (Hackett, 1976; Roberts and Buikstra, 2003: 101). In other examples of lesions, such as in the ischiopubic ramus and vertebral bodies T9 and L5, Buikstra's (1976: 355) description of tuberculous related skeletal lesions applies perfectly: 'concave, smooth-walled reaction areas that are oval, circular, or coalesced, ranging from 5 to 32 mm in diameter'. Overall, multifocal skeletal TB is the most likely diagnosis for PO21.

Molecular analyses revealed no evidence of the presence of TB aDNA in PO21. Nevertheless, negative aDNA results for the MTBC in PO21 alone is not sufficient to prove that PO21 was not infected with tuberculosis. In recent studies, 9 out of 56 (Müller et al., 2014a, b) and 3 out of 68 (Bos et al., 2014) individuals showing potential skeletal indicators of TB provided convincing MTBC aDNA results, using standard PCR or Next Generation Sequencing technologies, respectively. Increasing fragmentation and degradation of aDNA during the preservation history of PO21 might have contributed to the negative amplification results (Brown and Brown, 2011: 118–123).

4.2. Significance for bioarchaeology and paleoepidemiology of TB

This new Neolithic evidence of multifocal, disseminated skeletal TB, with its multiplicity of bony lesions, might be useful for understanding disease progression in the past, and possibly helping scholars in the diagnosis of other skeletons where the evidence is more limited/non-specific, and remains are incomplete (Wilbur et al., 2009; Roberts et al., 2009)

In the bioarchaeological literature, multifocal TB has been reported in a 4–5-year-old child from Predynastic Upper Egypt (AD S 500; 3,200 BC; Dabernat and Crubézy, 2010). Similarities with PO21 include spinal involvement, the destruction of an upper limb joint (radio-ulnar joint in AD S 500, gleno-humeral joint in PO21), and the presence of disseminated cyst-like lesions. PO21 does not display spinal collapse, but the precarious structural integrity of the spine can be inferred by the presence of a compression fracture in the vertebral body of the third lumbar vertebra. It is possible that the individual may have been on “bed rest” due to weakness before collapse of the spine could occur, although this can only be supposition. Contrary to AD S 500, PO21 does not show dactylitis (although not all hand phalanges were preserved), radiolucent lesions in the long bones (verified radiographically, results not shown here), or periosteal new bone formation on long bone diaphyses.

Evidence suggestive of disseminated skeletal TB is also present in children from the Roman period at Poundbury Camp, Dorset (1st–3rd Century AD, England; Lewis, 2011). In common with PO21 they displayed bilateral cribra orbitalia, and no spinal kyphosis is reported. However, contrary to PO21, alongside lytic lesions, they mostly show active new bone formation on lumbar and sacral vertebrae (interpreted as suggestive of gastrointestinal TB), and metaphyseal osteomyelitis (Lewis, 2011: 16). At Roman Pécs (4th Century AD, Hungary; Hlavenková et al., 2015), a 10-year-old child shows sharp angular kyphosis, lytic lesions on ribs,

and periosteal new bone formation on the femur, all occurrences that are absent in PO21. The bone deposition on the visceral aspects of their ribs was accompanied by a porosity not observed in PO 21 (cf. Fig. 3 in Hlavenkova et al., 2015:30 with Figures S1-14 and S1-15).

The earliest purported evidence in support of TB in a child (12 months old) is from Israel and dates to about 9,000 BP; skeletal evidence consisted of *serpens endocrania symmetrica* (SES) and *hypertrophic pulmonary osteoarthropathy* (HOA), both non-pathognomonic for TB. However, molecular analysis showed positive DNA and mycolic acid analyses for TB (Hershkovitz et al., 2008, 2015), but the data have been debated (see also Wilbur et al., 2009 and Donoghue et al., 2009 for discussion). PO21 does not show SES on the endocranial surface of the skull or HOA on the long bones (fragments of the left tibia of PO21, which are not visible in Figure 2, have been examined). Diaphyseal remodeling was in the form of osteoclastic activity on the left humerus, and new bone formation was visible only in the cortex overlying the epiphyseal lesion. Some new bone formation is present in the right supero-medial orbital surface, which might indicate scurvy; however, involvement of this area is atypical, and no other sign of infantile scurvy is present on the skeleton of PO21 (see Brickley and Ives, 2008: 56–67).

With the uniqueness of its pattern of skeletal lesions, the bone changes in the skeleton of PO21 confirm that the variety of clinical manifestations and disease progressions observed in people with skeletal TB today were clearly already present in the past (e.g. Burrill et al., 2007; Singh et al., 2009; Harishingani et al., 2010; Prapruttam et al., 2014; Elmi et al., 2013; Ye et al., 2016). At this stage of the research, it is difficult to determine whether this was due to different strains of the pathogen infecting the person, an effect of the host's immune response, or to environmental factors shaping how the pathogen affected the bones. Variability in individual TB-related skeletal manifestation is not necessarily surprising, especially considering the long history of host-pathogen coevolution indicated by DNA/aDNA studies; however, it makes difficult to discern pathognomonic lesions, or patterns of lesions, which may be used in bioarchaeological research for diagnosing TB.

At the current stage of research, similarly problematic is the understanding of the paleoepidemiological importance of TB for Ligurian Neolithic populations. The PO21 skeleton adds to those described by Formicola et al. (1987), and Canci et al. (1996); evidence from a fourth adult from Pollera Cave (PO22) with Pott's disease of the spine is under study. All burials were found in caves that are within a radius of 5 km of each other in the Finalese area, and

available dates span 7,500–6,500 years ago. A complete catalog and a precise chronological attribution for all the individuals from the Ligurian Neolithic has not been completed to date, and will be subject of future research; however, preliminary estimates suggest that at least two hundred individuals were excavated from the mid-1800s (partial catalogs in Parenti and Messeri, 1962; Benvenuti, 2012; Pagani, 2012). No other evidence has been published to date from the Neolithic of Italy (Rubini et al., 2014), and sites such as Çatalhöyük, with hundreds of burials, have not reported clear evidence of TB (Knüsel, personal communication 2016). Considering the potential for differential preservation of children's skeletons (Roberts and Buikstra, 2003: 50; Bello et al., 2006), the low percentage of untreated individuals with TB that show skeletal involvement today (1%, Turgut: 2001; 3–5%: Vigorita, 1999), and in particular of disseminated TB (4–5% of individuals with skeletal involvement, McTammany et al., 1963), one could speculate that TB must have had a high prevalence during the Neolithic in Liguria. Future research will aim to test this hypothesis, and will attempt to identify the possible risk factors for this population.

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Figure 1 – The geographical collocation in the Finalese area of Pollera Cave (1), where the burial of PO21 was discovered. In the lower right corner, a view of the upper chamber of the Pollera Cave (Attribution-Share Alike by Stefano Mazzone). The position of the main caves in the Finale Ligure area that yield Neolithic burials is also indicated: 2, Arene Candide; 3, Arma dell'Aquila; 5, Pian del Ciliegio; 6, Bergeggi (Maps from Google Maps™ and www.d-maps.com).

Figure 2 – The skeleton of Pollera 21, composite image.

Figure 3 – Visual summary of the most unambiguous pathological lesions in Pollera 21 (see text for detailed description). 1) Right scapula: clustered pitting and perforation of the cortical surface; 2) ribs: lytic activity and bone proliferation on the sternal ends and minimal bone deposition on the visceral surfaces; 3) left humerus: destruction of the proximal epiphysis and new bone formation; 4) os coxae: lytic lesion in the right auricular surface; destruction of the right ischio-pubic ramus with ample circular lesion; clustered pitting and perforation of the surface of the left ischium; 5) vertebral column: small or ample granulomatous lesions (see text) in the bodies of the fourth cervical vertebra, third and ninth thoracic vertebrae, third and fifth lumbar vertebrae; involvement of the neural arch in the eight thoracic and fifth lumbar vertebrae.

Figure 4 – Lateral and superior view of the proximal left humerus, showing deposition of new bone, the destruction of the surface in contact with the growth plate, and the underlying remodeled trabeculae.

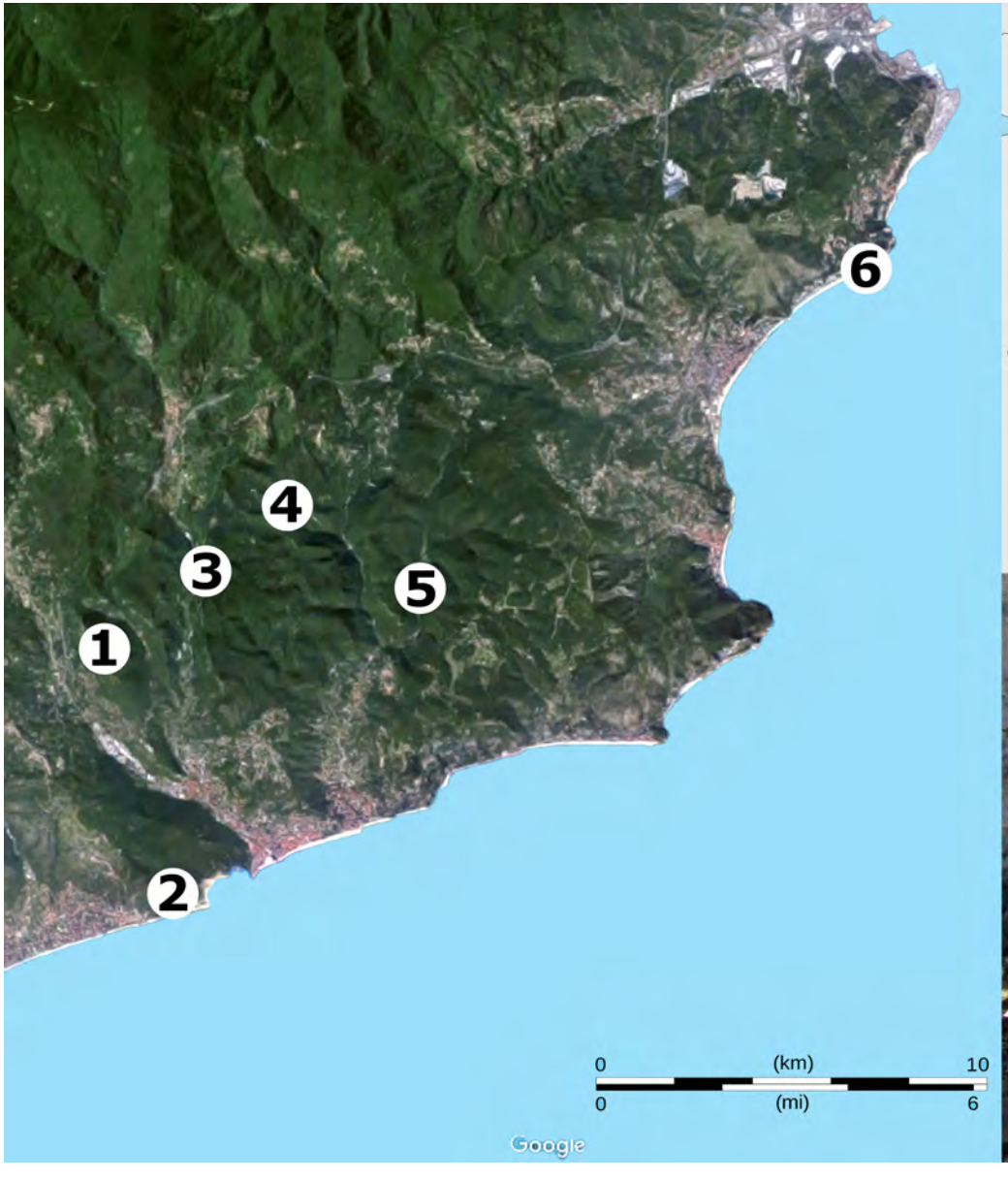
Figure 5 – Detail of the inferior margin of the right scapula, visceral view.

Figure 6 – Inferior view of the fourth cervical vertebral body.

Figure 7 – Inferior view of the ninth thoracic vertebral body.

Figure 8 – Sternal end of three ribs showing lytic lesions and proliferative bone processes. The first two ribs show the visceral aspect, the third displays the external aspect.

Figure 9 – Left, the right ischium showing the absence of the ischio-pubic ramus; right, anterior view of the lesion.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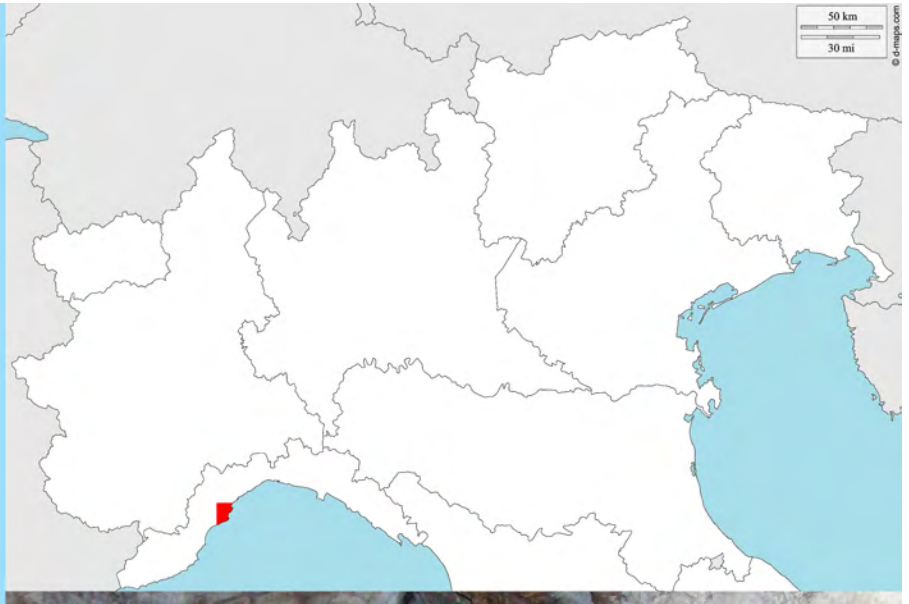
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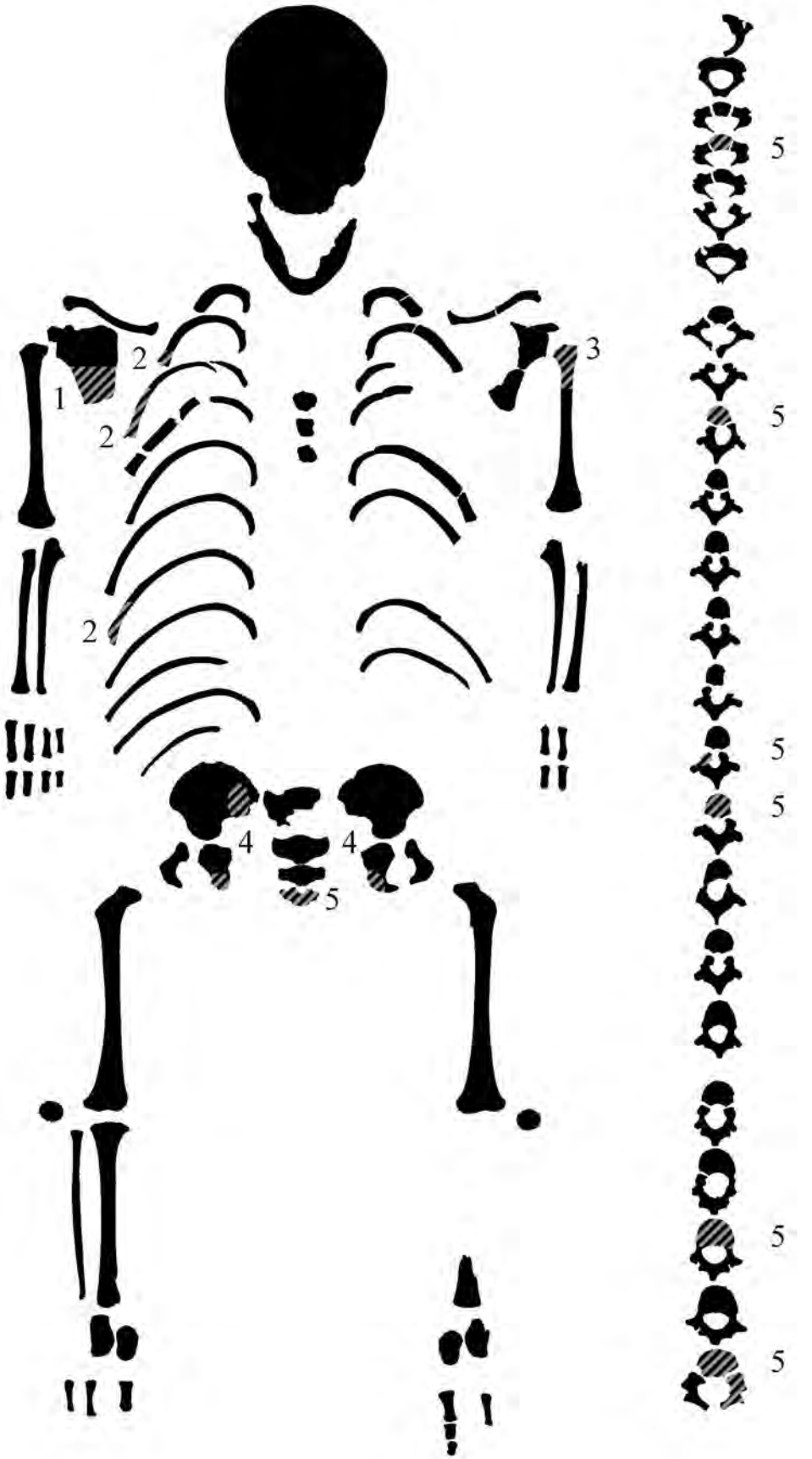
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SUPPLEMENTARY MATERIAL SI

Additional high-resolution pictures.

Figure S1-1 – The skeleton of PO21, composite image.

Figure S1-2 – The mandible of PO21, with taphonomic damage showing the maturation stage of the permanent molars.

Figure S1-3 – Bilateral cribra orbitalia in the orbital roof. Notice the bone deposition in the right orbit compared to encrustations of plaster with similar color in the left supraorbital margin.

Figure S1-4 – Anterior and posterior views of the humeri.

Figure S1-5 – Superior view the third thoracic vertebral body.

Figure S1-6 – Superior view of the ninth thoracic vertebral body.

Figure S1-7 – Anterior view of the eight thoracic neural arch.

Figure S1-8 – Inferior view of the third lumbar vertebra.

Figure S1-9 – Detail of the lesion in the third lumbar vertebra.

Figure S1-10 – Detail of the lesion in the third lumbar vertebra.

Figure S1-11 – Inferior view of the fifth lumbar vertebra.

Figure S1-12 – Detail of the lesion in the fifth lumbar vertebra.

Figure S1-13 – Superior view of the fifth sacral vertebra.

Figure S1-14 – Detail of the bone deposition in the visceral surface of a rib.

Figure S1-15 – Detail of the bone deposition in the visceral surface of a rib.

Figure S1-16 – Visceral view of the iliac bones.

Figure S1-17 – The left ischium with a detail of the posterior view.